

**117-Plat****Restricted Spots of Light Reveal an Efficacy Gradient of the Phototransduction Cascade Along the Rod Outer Segment**

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Rod photoreceptors consist of an outer segment (OS) and an inner segment (IS). Inside the OS a biochemical machinery transforms the absorption of photons into electrical signals (Yoshizawa and Wald, 1963; Baylor et al., 1979; Fung and Strayer, 1980). This biochemical machinery has been treated and thought to be in essence homogenous within OS with only marginal dishomogeneities (Lamb et al., 1981; Schnapf, 1983; Jarvinen and Lamb, 2005). In order to verify this assumption, we have used special optical fibres to deliver highly-localized light stimuli to OS. If a Gaussian beam of light is fed into an apertureless tapered optical fibers (TOF), coated with gold and titanium, the exiting spot of light does not have the usual lobes and is fairly restricted. As the TOF is moved from the OS base towards its tip, the amplitude of both the saturating response and the single-photon response decreased, demonstrating a pronounced reduction in the efficacy of the phototransduction machinery. We propose that this reduction in efficacy reflects a reduction in PDE levels along the OS, associated with discs aging. From measurements of the gradient of the saturating responses elicited by local flashes at different positions, we calculate that the longitudinal diffusion coefficient for cyclic guanosine monophosphate (cGMP) exceeds 500  $\mu\text{m}^2/\text{s}$ . As a result of this high diffusion coefficient, cyclic nucleotide-gated (CNG) channels near the tip of the outer segment are closed by diffuse flashes of light, despite the very low level of PDE in the discs in that region.

**118-Plat****Using Magnetic Probes to Study Receptor Clustering in Live Cells**

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<sup>1</sup>Nanobiophysics, University of Twente, Enschede, Netherlands, <sup>2</sup>Tumor Immunology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, <sup>3</sup>Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, <sup>4</sup>Nanoscale Biophysics, AMOLF, Amsterdam, Netherlands. During pathogen recognition T-Cell Receptors form microclusters which are believed to be the central signalling units. These structures could hold the secret behind the exceptional sensitivity of T-Cells in distinguishing single triggering 'agonist' peptides against a background of thousands. We have developed a biophysical approach based on magnetic tweezers that allows us to study the players involved in these receptor clusters and their dynamics. We use antibody functionalized magnetic beads to target CD3, a subunit of the TCR Complex to induce TCR clustering. Using magnetic tweezers, we move the beads along the cell membrane and simultaneously measure trafficking of co-receptors and proteins involved in the complex using confocal fluorescence microscopy and fluorescence recovery after photobleaching (FRAP). We study co-receptor CD6, which is considered a co-stimulator for cell activation during cluster formation. Our findings suggest that while CD6 is not physically associated with TCR complex, it gets recruited into the TCR clusters. There it is partially immobilized and moves along as clusters are displaced. The diffusion coefficient of CD6 is higher in bead-stimulated cells, whereas CD6 outside clusters diffuse faster than those within clusters. We are also downscaling this method to induce formation of receptor nanoclusters, in order to explore the effects of physical receptor oligomerization on the activity of TCR and Epidermal Growth Factor Receptors.

**119-Plat****Toward Single-Molecule Imaging of Electroporated Bacterial Flagellar Motor Proteins in Motile *E. coli***

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The bacterial flagellar motor is a membrane-spanning protein complex that functions as a rotary nano-machine to propel cells through liquid media. In *Escherichia coli*, it is powered by a transmembrane flux of  $\text{H}^+$  and the chemical energy is converted into work through a ring of stator units pushing on a central rotor. Bacterial chemotaxis is the biasing of movement towards regions that contain higher concentrations of beneficial, or lower concentrations of toxic, chemicals and is one of the most well-understood

sensory pathways. Upon phosphorylation, the response regulator protein CheY transduces changes of environmental chemical concentrations detected by specific transmembrane chemoreceptors to the flagellar motors: it binds to the N-terminus of the FliM proteins in the C-ring part of the motor (also known as the *switch complex*) inducing a cascade of conformational changes that modulate the direction of rotation. In this research, we combine a novel technique for protein internalization in live bacteria based on electroporation, recently developed by collaborators in the Department, and tracking analysis at the single-molecule level using a custom-built microscope. Our aim is to exploit electroporation of fluorescent dye-labelled chemotaxis and motor proteins in electrocompetent *motile E. coli* cells to perform an in-depth investigation of the interactions between the latter and the motor complex in vivo.

**120-Plat****Single-Molecule Tracking of Smoothened in the Primary Cilium**

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The Sonic Hedgehog (SHH) signaling pathway plays a key role in cell division and differentiation in embryonic development and adult stem cells. Malfunction in the pathway not only leads to deformities in developing embryos, but has been implicated in a variety of cancer types. The pathway is activated by the binding of SHH ligand to a transmembrane receptor protein, Patched, causing a cascade of translocation events involving the primary cilium, a small microtubule-structured organelle on the cell's surface whose spatial dimensions are on the order of the diffraction limit (~400 nm in diameter and 2-5  $\mu\text{m}$  long). One pathway intermediary, the transmembrane protein Smoothened (Smo) which accumulates in the ciliary membrane upon pathway activation, has proven to be susceptible to a number of small molecules that activate or disrupt the pathway; however, the detailed mechanisms underlying these effects remain unclear. By labeling Smo with an organic dye, we have performed single-molecule tracking experiments to elucidate its dynamics and interactions with high spatial resolution (~30 nm) and high temporal resolution (5-10 ms). We have observed that the primary mechanism for movement of Smo is diffusion with transient lingering in parts of the primary cilium consistent with binding events.

**121-Plat****Differential Clustering of Src Family Kinase on Lipid Bilayers Regulates a Net Phosphorylation Activity in a Receptor-Kinase-Phosphatase Network**

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Molecules required for T cell receptor (TCR) phosphorylation and T cell activation include a Src family kinase Lck that phosphorylates TCR and transmembrane phosphatase CD45. CD45 is required to dephosphorylate an inhibitory phospho-tyrosine of Lck to release its auto-inhibitory structure. In parallel, CD45 also dephosphorylates TCR and negatively regulates the signal, and thus spatial segregation of CD45 from TCR-enriched clusters in plasma membranes is thought to be important. To investigate whether these two modes of CD45 can be regulated in a single cell, we reconstitute and visualize the signal system by assembling recombinant signal proteins on planar lipid bilayer. We found that highly-clustered Lck massively auto-phosphorylates its inhibitory tyrosine. In these clusters, dephosphorylation of Lck by a small amount of CD45 had more profound effect for TCR phosphorylation than direct dephosphorylation of TCR by these CD45 molecules, resulting in a net positive effect of CD45 in TCR phosphorylation. In Jurkat T cells, similarly phosphorylated Lck clusters were observed. Altogether, we conclude that TCR phosphorylations in clusters could be enhanced by both excluding most CD45 molecules to reduce direct dephosphorylation and still including a small fraction of CD45 to positively regulate Lck activity, thus two modes of CD45 actions are not mutually exclusive but can be additive.

**122-Plat****Optical Probing of Metabotropic Glutamate Receptor Assembly and Cooperativity**

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Metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors that are found throughout the nervous system where they respond to the major